

5 transcription of a gene sequence, *i.e.*, a transcription-activating protein. "Transcription-activating" is a term used to refer to characteristics of a protein that promote transcription. As used herein, a transcription-activating protein would include proteins that increase accessibility  
 10 of the DNA to transcription complexes, for example, by opening or relaxing chromatin structure, proteins that promote the recognition and/or binding of transcription complexes to a target gene sequence, and/or proteins that promote transcription complex movement along the length of  
 15 the template DNA sequence.

Regulatory proteins of secondary metabolite production and the nucleic acid sequences encoding these are known to those skilled in the art. Non-limiting examples of regulatory proteins of secondary metabolite  
 20 synthesis include: regulator proteins of the aflatoxin/sterigmatocystin biosynthetic cluster (Woloshuk, C.P., *et al.*, *Appl, Environ. Microbiol.* **60**:2408-2414 (1994) and Brown, D.W., *et al.*, *Proc Natl Acad Sci U S A.* **93**:1418-1422 (1996)); regulator proteins of the paxilline  
 25 biosynthetic cluster (Young, C., *et al.*, *Mol, Microbiol.* **39**:754-764 (2001)); regulator proteins of the cephalosporin and penicillin biosynthetic clusters (Litzka O., *et al.*, *Antonie Van Leeuwenhoek* **75**:95-105 (1999); Schmitt E.K. and Kuck U., *J. Biol. Chem.* **275**:9348-9357  
 30 (2000); MacCabe *et al.* *Mol. Gen. Genet.* **250**:367-374 (1996); Suarez *et al.* *Mol. Microbiol.* **20**:529-540 (1996); Lambert *et al.* *Mol. Cell. Biol.* **17**:3966-3976 (1997); Su *et al.* *Genetics* **133**:67-77 (1993); regulator proteins of tricothecene synthesis (Trapp S.C., *et al.*, *Mol. Gen.*  
 35 *Genet.* **257**:421-432 (1998); Brown D.W., *et al.*, *Fungal Genet. Biol.* **32**:121-133 (2001); and Matsumoto G., *et al.* *Biosci. Biotechnol. Biochem.* **63**:2001-2004 (1999)); and regulator proteins of lovastatin synthesis (Kennedy, J., *et al.*, *Science* **284**:1368-1372 (1999); Hendrickson *et al.*,  
 40 *Chem. Biol.* **6**:429-439 (1999) Tag, A. *et al.*, *Mol Microbiol.* **38**:658-65 (2000)).

- 5 Certain embodiments of the aspects of the invention disclosed herein relate to the lovE regulator protein, a protein which plays a key role in the biosynthesis of lovastatin. More particularly, certain embodiments of the aspects of the invention relate to variant proteins of the
- 10 lovE regulator protein and methods of making the same. Such proteins are variant with respect to the following A. *terreus* wild-type *lovE* sequences (SEQ ID NOS:91 and 92).

**Table 1: Amino Acid and Nucleic Acid Sequences of Wild-type *lovE***

**Wild-type *lovE* Amino Acid Sequence**

maadqgiftnsvtlspvegsrtggtlprrafrsrscdrchaqkikctgnkevtgrapcgrc  
qqaglrvcysercpkrklrqsraadlv sadpdpclhmssppvpssqlpldvsseshsnts  
rqfldppdsydwswtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekap  
lppvssdiaraasaqrelfddlsavsqeleellavtvewpkgeiwthpigmffnasrrl  
ltvlrqqaqadchqgtldeclrtnlftavhcyilnvriltaiselllsqirrtqnshms  
plegsrsqspsrddtssssghssvdtipffsenlpigelfsyvdpplthalfsacttlhvg  
vqllreneitlgvhsaaggiaasismssgepgediartgatnsarceegpttpaarvlfmfl  
sdegafqeaksagsrgrtiaalrrcyedifslarkhkhgmlrdlnnipp (SEQ ID  
NO:91)

**Wild-type *lovE* DNA Sequence**

atggctgcagatcaaggtatattcacgaactcggtcactctctcgcacagtggagggttca  
cgcaccggtggaacattaccccgccgtgcattccgacgctcttgtgatcgggtgcatgca  
caaaagatcaaagtactggaaataaggaggttactggccgtgctccctgtcagcgttgc  
cagcaggctggacttcgatgcgtctacagtgcgcgatgccccaaagcgcaagctacgcaa  
tccagggcagcggatctcgtctctgctgacccagatccctgcttgacatgtcctcgct  
ccagtgccttcacagagcttgccgctagacgtatccgagtcgcattcctcaaatacctcc  
cggcaatttcttgatccaccggacagctacgactggctcgtggacctcgattggcactgac  
gaggctattgacactgactgctgggggctgtcccaatgtgatggaggcttcagctgtcag  
ttagagccaacgctgccgatctaccttcgcccttcgagctctacggttgaaaaagctccg  
ttgccaccggtatcgagcgcacattgctcgtgcggccagtgcgcaacgagagcttttcgat  
gacctgtcggcgggtgtcgcaggaactggaagagatccttctggccgtgacggtagaatgg  
ccgaagcaggaaatctggaccatcccatcggaatgtttttcaatgcgtcacgacggctt  
cttactgtcctgcgccaacaagcgcaggccgactgccatcaaggcacactagacgaatgt  
ttacggaccaagaacctctttacggcagtagactgttacatattgaatgtgcggattttg  
accgccatatacgagttgctcctgtcgcaaataggcggaccacagacacagccatatgagc  
ccactggaaggagtcgatccagtcgccgagcagagacgacaccagcagcagcagcggc  
cacagcagtggtgacaccataaccttcttttagcgagaacctccctattggtgagctgttc  
tcctatgttgacccctgacacacgccttattctcggcttgcaactacgttacatgttggg  
gtacaattgctgcgtgagaatgagattactctgggagtagactccgccaggggcattgca  
gcttccatcagcatgagcggggaaccaggcgaggatatagccaggacagggggcgaccaat  
tccgcaagatgcgaggagcagccgaccactccagcggctcgggttttgcattgttcttg  
agtgatgaaggggctttccaggaggcaaagtctgctggttcccagggtcgaaccatcgca  
gcactgcgacgatgctatgaggatatcttttccctcgcccgcaaacacaaacatggcatg  
ctcagagacctcaacaatattcctccatga (SEQ ID NO:92)

- 15 As used herein, the term "secondary metabolite" means a compound, derived from primary metabolites, that is produced by an organism, is not a primary metabolite, is not ethanol or a fusel alcohol, and is not required for growth under standard conditions. Secondary metabolites

5 are derived from intermediates of many pathways of primary  
metabolism. These pathways include, without limitation,  
pathways for biosynthesis of amino acids, the shikimic  
acid pathway for biosynthesis of aromatic amino acids, the  
polyketide biosynthetic pathway from acetyl coenzyme A  
10 (CoA), the mevalonic acid pathway from acetyl CoA, and  
pathways for biosynthesis of polysaccharides and  
peptidopolysaccharides. Collectively, secondary  
metabolism involves all primary pathways of carbon  
metabolism. Particularly preferred in embodiments of the  
15 aspects of the invention are fungal secondary metabolites  
(See, Fungal Physiology, Chapter 9 (Secondary(Special)  
Metabolism), Griffin, D. H., John Wiley & Sons, Inc.;  
ISBN: 0471166154).

20 "Secondary metabolite" also includes intermediate  
compounds in the biosynthetic pathway for a secondary  
metabolite that are dedicated to the pathway for synthesis  
of the secondary metabolite. "Dedicated to the pathway  
for synthesis of the secondary metabolite" means that once  
the intermediate is synthesized by the cell, the cell will  
25 not convert the intermediate to a primary metabolite.

"Intermediate compounds" also include secondary metabolite  
intermediate compounds which can be converted to useful  
compounds by subsequent chemical conversion or subsequent  
biotransformation. As such, providing improved  
30 availability of such intermediate compounds would still  
lead to improved production of the ultimate useful  
compound, which itself may be referred to herein as a  
secondary metabolite. The yeast *Saccharomyces cerevisiae*  
is not known to produce secondary metabolites.

35 The term "primary metabolite" means a natural product  
that has an obvious role in the functioning of almost all  
organisms. Primary metabolites include, without  
limitation, compounds involved in the biosynthesis of  
lipids, carbohydrates, proteins, and nucleic acids. The  
40 term "increasing the yield of the secondary metabolite"  
means increasing the quantity of the secondary metabolite  
present in the total fermentation broth per unit volume of  
fermentation broth or culture.